Indirect evidence of nasal inflammation assessed by titration of inflammatory mediators and enumeration of cells in nasal secretions of patients with chronic rhinitis

Jalel Knani, MD,[a] Alison Campbell, PhD,[a] Ingrid Enander, PhD,[b] Christer G. B. Peterson, DMSci,[b] François-Bernard Michel, MD,[a] and Jean Bousquet, MD, PhD[a] *Montpellier-Cedex, France, and Uppsala, Sweden*

Pathophysiologic mechanisms of perennial rhinitis are poorly understood. The characterization of inflammation was studied in nasal lavage of patients with perennial rhinitis by the enumeration of cells involved in the allergic inflammation and the measurement of six mediators released in nasal secretions to determine whether some mediators were relevant for the etiologic diagnosis and the occurrence of symptoms. Ten healthy subjects and 57 patients with perennial rhinitis were placed into four groups according to the symptoms they presented at the time of the study and the origin of the allergy. Allergy was characterized by the history, skin prick tests to standardized allergens, and RAST. Eosinophil protein X (EPX), tryptase, histamine, myeloperoxidase, prostaglandin D_2, and leukotriene C_4/D_4 (LTC_4/D_4) were measured in nasal lavage by enzyme assay or radioimmunoassay. Eosinophils and neutrophils were enumerated after cytocentrifugation of the lavage fluid and May Grunwald Giemsa staining. Tryptase, myeloperoxidase and EPX but not histamine levels were increased in all four patient groups. Eosinophils, LTC_4/D_4, and prostaglandin D_2 were significantly ($p < 0.001$, $p < 0.03$, and $p < 0.01$) increased in allergic and symptomatic patients. EPX was significantly increased in symptomatic allergic and nonallergic patients. This study suggests the involvement of mast cells, neutrophils, and eosinophils, but the latter cells appear to have a more prominent role. The importance of EPX and LTC_4/D_4 in the characterization of chronic symptomatic rhinitis was also observed. (J ALLERGY CLIN IMMUNOL 1992;90:880-9.)

Key words: *Rhinitis, inflammation, allergy, eosinophils, neutrophils, histamine, tryptase, PGD_2, LTC_4*

The pathophysiologic mechanisms underlying nasal allergic diseases have largely been studied in pollen allergy.[1, 2] The studies of mediator release after nasal allergen challenge pioneered by Naclerio et al.[3] made it possible to analyze the events of the allergic reaction more precisely. Cells and mediators occurring during the early- and late-phase reactions,[4, 5] as well as during rechallenge,[6] have shown that the allergic inflammation caused by the coupling of mast cell–bound IgE to specific allergens leads to the release of vasoactive (histamine,[3, 6-8] sulfidopeptide leukotrienes,[8-12] prostaglandin D_2 [PGD_2][3, 6, 7] or kinins[3, 6, 13]) and chemotactic mediators,[9] tryptase,[14, 15] eosinophil-derived

Abbreviations used

A S+:	Allergic and symptomatic
A S−:	Allergic and symptom-free
NA S+:	Nonallergic symptomatic
NA S−:	Nonallergic symptom-free
ECP:	Eosinophil cationic protein
EPX:	Eosinophil protein X (eosinophil-derived neurotoxin)
LTC_4/D_4:	Leukotriene C_4/D_4
NARES:	Nonallergic rhinitis with eosinophils syndrome
PGD_2:	Prostaglandin D_2

From the Clinique des Maladies Respiratoires, Hopital l'Aiguelongue, Centre Hospitalier Universitaire,[a] Montpellier-Cedex, and Pharmacia Diagnostics AB,[b] Uppsala.

Received for publication Dec. 30, 1991.
Revised July 29, 1992.
Accepted for publication July 30, 1992.

Reprint requests: Jean Bousquet, MD, Clinique des Maladies Respiratoires, Hopital l'Aiguelongue, Centre Hospitalier Universitaire, 34059-Montpellier-Cedex, France.

1/1/41434

granule proteins,[16-18] and the mucosal infiltration by metachromatic cells,[20, 21] eosinophils,[21-24] and possibly neutrophils.[16] However, nasal challenge does not completely represent the natural pollen exposure, and several studies that have examined the occurrence of mediators and cells in nasal secretions collected during the pollen season have often, but not always confirmed the activation of mast cells and eosinophils.[15, 21-35]

Perennial rhinitis is a more complex disease, including allergic rhinitis, the nonallergic rhinitis with eosinophilia syndrome (NARES) and the loosely defined vasomotor rhinitis.[36-39] Symptoms caused by allergic rhinitis or the NARES are similar to rhinorrhea, nasal obstruction, sneezing, and pruritus. On the other hand, in patients with vasomotor rhinitis the major symptom is nasal obstruction.[40] Nasal inflammation has only been examined in few studies, and it has been observed that patients have an inconstant nasal eosinophilia even in allergic rhinitis.[35, 41] The release of mediators in nasal secretions has been examined in one study, and histamine was not found to be increased.[42]

Some of the cells involved in the allergic inflammation and release of specific mediators include eosinophils that release granule constituents such as major basic protein and eosinophil cationic protein (ECP) and eosinophil protein X (EPX),[43-45] mast cells releasing histamine and tryptase,[46-49] and neutrophils releasing myeloperoxidase, which is stored in the azurophil granule and released during phagocytosis or cell activation.[50] On the other hand, sulfidopeptide leukotrienes and PGD_2 are released by a variety of cells, including mast cells.[51, 52]

The characterization of inflammation was studied in nasal lavage of patients suffering from perennial rhinitis by the enumeration of cells involved in the allergic inflammation and the measurement of mediators released in nasal secretions. Six mediators were selected because of their relevance to studies of nasal challenge or their importance during the pollen season: EPX, tryptase, histamine, myeloperoxidase, PGD_2, and sulfidopeptide leukotrienes. Four groups of patients with perennial rhinitis were studied according to the symptoms they presented at the time of the study and the origin of the allergy to determine whether some mediators were relevant for the etiologic diagnosis and the occurrence of symptoms. The major goal of the study was the measurement of mediators in nasal secretions, and the second goal was the enumeration of cells. This is why we deliberately used nasal lavage to recover cells rather than an alternative method such as the brush method,[53] which might modify the release of mediators.

TABLE I. Symptom score of chronic rhinitis

Sneezing	
Occasional	1
Common	2
Common and usually >5	3
Rhinorrhea	
Anterior	1
Posterior	1
Both symptoms	3
Blockade	
Patient can breathe freely	0
Patient can only breathe with difficulty	1
One nostril is blocked	2
Both nostrils are blocked	3
Pruritus	1

MATERIAL AND METHODS

Subjects

Fifty-seven patients (26 men, age: 31.3 ± 16.4 years) volunteered to participate in the study after informed consent was given. All had symptoms of perennial rhinitis characterized by anterior rhinorrhea and nasal obstruction, inconstant sneezing, and nasal pruritus. Eighteen also had symptoms of conjunctivitis. The duration of symptoms had ranged from 2 to 30 years. Thirty-three patients had current symptoms, and 24 had had symptoms between 1 to 10 days before the study. None of the patients had received any form of specific immunotherapy or had acute or chronic sinusitis.

Ten healthy volunteers (22 to 45 years old; mean ± SD, 30.0 ± 4.1 years) were used as a control group. They were nonallergic and had never suffered from seasonal or perennial rhinitis.

It was important that none of the subjects had had any nasal infection within the previous month, since histamine and other mediators may be elevated in nasal secretions.[54] Patients were excluded from the study if they had taken systemic corticosteroids of any form during the past 2 months, topical corticosteroids the previous month, sodium cromoglycate, H_1-blockers, or ketotifen the week before the test. None of the patients had been treated with astemizole.

The study was conducted after informed consent had been obtained from the participants and after approval of the study by the ethics committee of the hospital.

Investigations

Rhinitis score. The clinical severity of rhinitis was quoted according to a symptom score previously used in seasonal rhinitis (Table I).[55] The clinical score of rhinitis was filled in by a single investigator (J.K.) who performed the whole clinical study.

Etiologic investigations of rhinitis. All patients underwent identical investigations. Allergy tests, including a battery of extracts of common food and aeroallergens found

in the Montpellier area,[56] were skin prick tests performed according to a technique previously described in detail elsewhere.[57] Total serum IgE (Phadebas PRIST; Pharmacia Diagnostics AB, Uppsala, Sweden) and Phadiatop (Pharmacia) completed the study. Serum-specific IgE was titrated by the Phadebas RAST (Pharmacia) in patients with positive skin prick tests. All patients had sinus radiography.

Nasal washing. A wash with 5 ml of saline solution in each nostril was performed. The wash fluid was immediately centrifuged at +4° C for 15 minutes at 15,000*g*, and the sol phase was separated from the gel phase by use of a pipette and stored at −20° C until assay was performed.

Examination of nasal lavage cells. The nasal cytology was performed on cytocentrifuged preparations (Shandon, U.K.) stained with use of May Grundwald Giemsa according to the method of Pipkorn and Karlsson.[58] Cells were only enumerated when at least 50 cells could be counted.

Titration of mediators in nasal washing. ECP was not titrated, because in a pilot study we observed that when we used a double antibody radioimmunoassay (Pharmacia), its levels were not often increased in nasal lavage fluids of patients with allergenic perennial rhinitis. Instead, we used EPX as a marker of eosinophils. It was titrated by means of a double-antibody radioimmunoassay (Pharmacia) with a polyclonal rabbit antibody as previously described by Carlson et al.[59] EPX in samples competes with a fixed amount of iodine 125 (^{125}I)–labeled EPX for the binding sites of specific antibodies. The EPX standards are calibrated against pure EPX prepared according to Peterson and Venge.[60] Levels under 0.7 μg/L are undetectable. The interassay coefficient of variation was under 10%. Cross-reactivity of the assay with ECP from eosinophils was <0.03%.

Tryptase was titrated in unconcentrated nasal lavage with use of a commercially available kit (Pharmacia). In brief, the assay is a solid-phase radioimmunoassay based on two tryptase-specific monoclonal antibodies.[61, 62] In the assay the tryptase in the sample reacts with antitryptase antibody bound to the wall of the test tube. Then ^{125}I-antitryptase is added to form a labeled complex so that tryptase in the sample reacts simultaneously with the solid phase antitryptase bound to the test tube and ^{125}I-antitryptase forming an antitryptase-tryptase ^{125}I-antitryptase complex. After an overnight incubation at room temperature, the tubes were washed three times, and the remaining radioactivity was determined. The intraassay and interassay coefficients of variation are less than 4% for samples. Levels of tryptase under 0.5 μg/L are undetectable,[63] and the tryptase standards are calibrated against pure tryptase prepared with use of the method of Schwartz et al.[64]

Myeloperoxidase was titrated with use of a double antibody radioimmunoassay (Pharmacia). Myeloperoxidase in samples competes with a fixed amount of ^{125}I-labeled myeloperoxidase for the binding sites of a specific polyclonal rabbit antibody. The technique used was as stated on the package insert. The interassay coefficient of variation was under 12%, and levels under 8 μg/L are undetectable.

Histamine was titrated by radioimmunoassay with a monoclonal antibody against acylated-histamine (Immunotech, Marseille, France),[65] and the limit of detectability is 0.05 ng/ml. Since in some studies the levels of histamine were not found to be increased after nasal challenge with allergen or in studies performed in seasonal or perennial rhinitis,[7, 8, 23, 25, 42] it is possible that histamine might have been degraded into methylhistamine, which is not recognized by the monoclonal antibody against histamine used in the present study. We therefore verified the validity of our histamine assay in 30 samples by comparing the titration of histamine using the Immunotech technique with that of methylhistamine and histamine by use of a commercially available kit (Pharmacia), and we observed a strong correlation between both assays ($r_s = 0.85, p < 0.001$, Spearman rank test). This experiment is consistent with previous comparative studies and confirms the validity of the assay used in the present study.[66, 67]

PGD_2 was assayed by enzyme immunoassay according to the method of Maclouf et al.,[68] and acetylcholinesterase from electric eels was the enzyme used in the assay.[69] The technique used, which is commercially available (Stallergènes Laboratories, Fresnes, France), was described in detail in a previous article.[7] The limit of detectability is 30 pg/ml.

Leukotriene C_4/D_4 (LTC_4/D_4) was assayed by enzyme immunoassay with use of a commercially available kit (Stallergènes Laboratories), and acetylcholinesterase from electric eels was the enzyme used in the assay.[70] The antibody used in this assay shows a cross-reactivity at 50% B/B_0 of 46% with LTD_4 and 2% with LTE_4 at +22° C. The limit of detectability is 15 pg/ml.

Design of the study

Classification of patients. Patients were classified as "allergic" if they had (1) a history suggestive of perennial rhinitis throughout the year with an exacerbation during the late summer and autumn, when mite and mold counts are at the higest in our area, or if they had symptoms for more than 4 months during the late summer and autumn, and (2) positive skin tests and RAST to house dust mites and/or molds. Patients were classified as "nonallergic" if they had (1) a history suggestive of perennial rhinitis for over 4 months without any exacerbation during the late summer and autumn, (2) no positive skin test to perennial allergens including house dust mites and molds, and (3) a negative Phadiatop outcome. Only patients with these clear-cut characteristics were entered in the study. Patients were classified into four groups according to the origin of allergy and the symptoms they presented on the day of the study: allergic rhinitis and current symptoms (A S+), allergic rhinitis without current symptoms (A S−), nonallergic rhinitis with current symptoms (NA S+), and nonallergic rhinitis without current symptoms (NA S−). The study was carried out between October and December, at a time when pollens are found in insignificant amounts in the Montpellier area.

Statistical analysis. Statistical analyses were carried out by means of nonparametric tests. The Kruskal-Wallis test was used to compare all groups, and the Mann-Whitney U test was used for individual group comparisons. Spearman

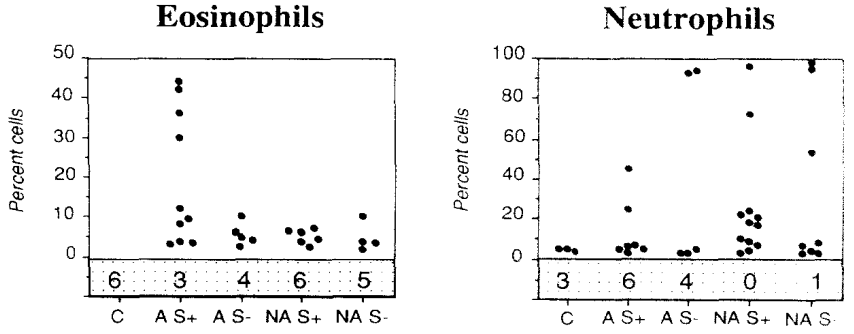

FIG. 1. Cells recovered by nasal lavage. Only patients with enumerated slides are presented. Numbers indicate patients with undetectable eosinophils or neutrophils.

TABLE II. Global results

		A S+	A S−	NA S+	NA S−	Control
No. of subjects		19	13	14	11	10
Age (yr)		23.2 ± 12.5	24.6 ± 11.8	38.4 ± 13.6	44.5 ± 20.2	30.0 ± 4.1
Symptoms						
Rhinorrhea	m ± SD	2.1 ± 0.9	1.9 ± 0.3	1.7 ± 0.5	2.4 ± 0.5	0
	% patients	100	100	100	100	
Obstruction	m ± SD	1.7 ± 0.5	1.6 ± 0.7	1.6 ± 0.6	1.2 ± 0.5	0
	% patients	100	100	100	91	
Pruritus	m ± SD	0.6 ± 0.6	0.7 ± 0.5	1.0 ± 0.8	0.4 ± 0.5	0
	% patients	58	77	86	100	
Sneezing	m ± SD	1.8 ± 1.0 (a)	0.6 ± 0.8 (b)	0.7 ± 0.6 (c)	0.3 ± 0.5	0
	% patients	90	54	64	46	
Conjunctivitis	% patients	47%	73%	0	0	0
% Patients with cells in nasal lavage						
Cells enumerated		68.4	69.2	85.7	81.9	60
Eosinophils*		82.1	55.6	33.3	44.4	0
Neutrophils*		53.8	55.6	100	81.3	50

p a/b < 0.01, p a/c < 0.01 (Mann-Whitney U test).
*Percentage in patients with enumerated cells.

rank correlations were not performed when the levels of a mediator were undetectable in more than 50% of samples. Results are given in mean or median ± standard deviations.

RESULTS

Characteristics of the patients

Mean ages of patients with rhinitis and healthy subjects were not significantly different. However, patients with allergic rhinitis (A S+ and A S−) were significantly younger ($p < 0.005$, Mann-Whitney U test) than those with nonallergic rhinitis (NA S+ and NA S−) (Table II). All allergic patients had a house dust mite allergy assessed by positive skin tests and RASTs. Other sensitivities included molds (6 patients), animal danders (7 patients), and 12 patients had both perennial and seasonal symptoms as a result of pollen allergy.

Five patients in the A S− group and two patients in the A S+ group had symptoms that subsided during the summer. All nasal symptoms except sneezing were equally distributed among the four groups of patients (Table II). In particular, obstruction was not more severe in the nonallergic group. Sneezing was significantly more severe in A S+ patients than in A S− or in NA S+ patients ($p < 0.01$, Mann-Whitney U test). Only patients in the allergic group (A S+ and A S−) had conjunctivitis.

Nasal lavage cells

A readable cytocentrifuge slide was obtained on 68.2% to 85.7% of patients and 60% of control subjects. Eosinophils were only found in patients with rhinitis. In the A S+ group eosinophils were enumerated in 82.1% of readable slides. In the other three

TABLE III. Statistical evaluation of the data

	Control subjects vs patients with rhinitis				Allergic vs nonallergic	
	A S+	A S−	NA S+	NA S−	A S+/NA S+	A S−/NA S−
Tryptase	0.007	0.005	0.005	0.005	NS	NS
Histamine	NS	NS	NS	NS	NS	NS
EPX	0.003	0.01	0.01	0.05	0.01	NS
MPO	0.01	0.01	0.01	0.01	NS	NS
LTC_4	0.01	NS	NS	NS	NS	NS
PGD_2	0.01	NS	NS	NS	NS	NS
Neutrophils	NS	NS	0.03	0.05	0.02	NS
Eosinophils	0.03	NS	NS	NS	0.04	NS

Statistical analysis by Mann-Whitney U test.

groups of patients they were enumerated in 33.3% to 56.5% (Table II). By Kruskal-Wallis test there was no significant difference between groups for any of the cell types studied. The percentage of eosinophils was significantly greater in the A S+ group than in the three other groups of patients (Fig. 1, Table III). Neutrophils were enumerated in 55.6% to 100% of patients with readable slides, and the greatest numbers were found in nonallergic individuals (Fig. 1, Table III). However, only eight patients had neutrophils over 25% of enumerated cells. Neutrophils were significantly increased in the NA S+ and NA S− groups, and these cells appeared to present a normal morphologic make up.

Mediator levels in nasal lavage fluid

Levels of mediators and statistical analyses are given in Tables II and III and Fig. 2. By Kruskal-Wallis tests a significant difference occurred between groups for EPX ($p < 0.0001$) and LTC_4/D_4 ($p < 0.0001$).

Tryptase levels were undetectable in all the normal individuals but were detected in all but five patients. Levels ranged from undetectable to 1.9 μg/L. A significant increase occurred in tryptase levels in all four groups of patients, but no significant difference occurred between the groups. Histamine levels were detectable in all patients and subjects tested, and no significant difference occurred between groups.

EPX was detectable in four control subjects and most patients with chronic rhinitis ($p < 0.002$, Mann-Whitney U test). Moreover, although some overlap exists between groups of patients with chronic rhinitis, statistical differences exist between A S+ and A S− groups ($p < 0.05$, Mann-Whitney U test) and A S+ and NA S+ patients ($p < 0.01$, Mann-Whitney U test).

Myeloperoxidase levels were undetectable in control subjects and in 50% of patients. A significant difference was observed between control subjects and patients with rhinitis ($p < 0.005$, Mann-Whitney U test). However, no significant difference occurred between the four groups of patients.

LTC_4/D_4 levels were undetectable or low in the control group. They were significantly increased in the A S+ group in comparison with control subjects ($p < 0.01$, Mann-Whitney U test) and the A S− group ($p < 0.01$, Mann-Whitney U test). More than 70% of symptomatic patients (A S+ and NA S+) had LTC_4/D_4 levels over the highest level found in the control group. PGD_2 levels were low, under 10 ng/L in the control group and significantly higher in the A S+ group ($p < 0.01$, Mann-Whitney U test).

Correlations between parameters

In patients with rhinitis a significant correlation was observed between LTC_4/D_4 and EPX ($r_s = 0.41$, $p < 0.005$, Spearman rank correlation). No other significant correlation was observed.

DISCUSSION

This study examined nasal lavage cells and six inflammatory mediators released in nasal secretions of four groups of patients with perennial rhinitis and a control group. Patients with symptomatic allergic rhinitis had a significant increase of eosinophils in nasal secretions as well as an increase of EPX, LTC_4/D_4 and to a lesser extent PGD_2. Patients with nonallergic rhinitis, whether or not they were symptomatic, presented an increase in neutrophils and EPX. Tryptase, and neutrophil myeloperoxidase were increased in all rhinitis groups.

Nasal inflammation can be studied by different methods. Nasal biopsies directly identify inflammatory cells and mucosal damage,[31-35, 70] but they usually give little information on the activation state of the cells, cannot give quantitative results, and do not discern the possible heterogeneity of mucosal lesions.

Symptomatic vs nonsymptomatic		Allergic vs nonallergic
A S+/A S−	NA S+/NA S−	
NS	NS	NS
NS	NS	NS
0.05	NS	0.008
NS	NS	NS
0.01	NS	NS
NS	NS	NS
NS	NS	0.03
NS	NS	NS

Hence, in this study we used nasal lavage because it indirectly identifies nasal inflammation by the enumeration of cells and measurement of mediators, provides information on a more extensive area of the nose, and is quantitative. However, some mediators may be degraded, and even if an increased level of a specific mediator is found in lavage fluid, the concentration of this mediator at the site of its action and whether it really participates in the reaction is unknown.[28] Even less is known about whether certain mediators and/or cytokines act in combination, increasing or decreasing the response of the others.[1] However, a mediator in nasal secretions may be considered as a marker of activity of a specific cell. This appears to be the case for EPX (eosinophils),[44, 45, 60] histamine (basophils and mast cells),[46] tryptase (mast cells),[47-49] and myeloperoxidase (neutrophils).[50] We did not concentrate the lavage fluid. Although this may have led to an increase in the detectability of certain mediators such as myeloperoxidase, concentration is not completely quantitative. The enumeration of cells may be improved by use of the Rhinoprobe (Rhino-Technics, San Diego, Calif.),[53] but this might modify in turn the release of mediators. Moreover, obtaining three lavages may also increase the yield of the cells. Finally, the analysis of inflammatory cells requires both the quantitation of the total cell number as well as the differential cell count. In the present article we only characterized the differential pattern, and the lack of clear discrimination between the different rhinitis groups may be related with the fact that we did not give cell counts.

The assessment of the clinical score of rhinitis was based on symptoms commonly occurring in rhinitis and on our experience of seasonal allergic rhinitis. In fact, as for many other allergic diseases there is no accepted symptomatic score that has been previously validated. Patients answered a questionnaire on the day of the nasal lavage, and there may be some biases

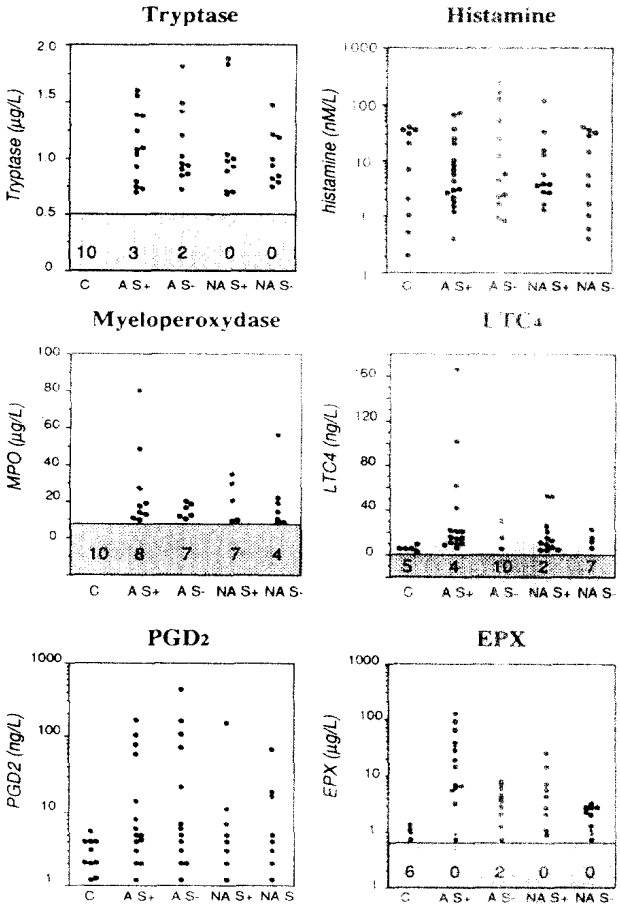

FIG. 2. Levels of mediators in nasal secretions. *C*, Control subjects. Numbers indicate patients with undetectable mediator.

for patients who were symptom-free (A S − and NA S −) in describing their symptoms. However, because we wanted to analyze the inflammatory events in all four groups of patients there was no other option. The discrimination between allergic and nonallergic patients was clear-cut, because all patients about whom we had questions were withdrawn from the study. As has already been reported patients with allergic rhinitis were significantly younger than those with nonallergic rhinitis. In the latter group, although anterior rhinorrhea was a common symptom, only a subset of patients had the so-called NARES,[36, 37] since eosinophils were found in approximately one third of these patients.

The original features of this paper are (1) the study of untreated, perfectly characterized patients with perennial rhinitis, (2) the study of symptomatic and symptom-free patients in an attempt to find out whether there might be a mediator that would differentiate these two groups of patients, (3) the use of

many mediators relevant to the allergic inflammation, and (4) the use of EPX as a marker of eosinophil activation.

One of the pitfalls that might be raised by this study is the lack of diagnosis of allergic patients. It is generally accepted that prick tests are less sensitive, less reproducible, but more specific than intradermal tests, thus the value of prick tests is limited by low potency extracts inducing false-negative results. With standardized extracts the prick test appears to be sensitive enough, and this method has been recommended even for research purposes. It is considered that prick test correlates better with symptoms, although in patients with a low sensitivity, intradermal skin tests may be the only positive test. All patients had a Phadiatop to confirm the results of skin prick tests. Moreover, in our area more than 95% of the patients with chronic allergic symptoms are sensitized to one or more allergen extracts that are in standardized form, and the Phadiatop is positive in more than 87% of patients allergic to perennial allergens (unpublished data) so that the possibility of missing an allergic patient is small. Thus overlap in nasal findings in the different groups was analyzed by Kruskal-Wallis test and might reflect in part some "low sensitivity" allergic patients included in the population with negative prick test outcomes. Using intradermal skin tests we might have identified some of the patients with negative prick tests. Another difficulty in the characterization of the patients is selection of asymptomatic patients. It is clear that asymptomatic inflammation may persist for some days after allergic or nonallergic triggers. This can be easily demonstrated in asthma with use of nonspecific hypersensitivity tests. Thus residual inflammatory changes in the nasal mucosa of individuals recently, but not currently, symptomatic may have led to overlaps in nasal findings in the symptomatic and asymptomatic groups.

The role of eosinophils in rhinitis has been demonstrated in seasonal and perennial rhinitis,* but although a time relationship appears to exist between the increase in eosinophils and levels of ECP or major basic protein and the development of symptoms of a late-phase reaction after allergen challenge, no individual relationship between these factors has been found.[16, 35] In the present study eosinophils were significantly increased in the A S+ group, confirming the importance of these cells in allergic perennial rhinitis. EPX was found to be of great value because most patients had detectable levels in contradistinction to normal individuals, and A S+ patients had the greatest levels. This study therefore confirms the role of eosinophils in allergic and nonallergic rhinitis. The measurement of EPX may be of greater value than the enumeration of eosinophils in the diagnosis of chronic rhinitis, but the number of patients is too low in the present study to make definite conclusions.

Histamine is inconstantly released during the immediate- and the late-phase reactions after allergen challenge.[2, 8-10, 15, 20, 26] It does not always correlate with the occurrence of symptoms,[8, 9] and the levels of pharmacologically active histamine in nasal secretions may be very high in asymptomatic patients before any challenge and may be increased during viral infections.[8, 9, 30] During allergen challenge, the release of histamine does not appear to be significantly correlated with those of tryptase.[15] During the pollen season, levels of histamine in nasal secretions were found to be similar than before in some studies,[31] and one study of perennial rhinitis found that histamine levels were not increased.[42] In the present study we did not find elevated levels of histamine in nasal secretions of any of the groups of patients, whereas tryptase was elevated in all these four groups. This discrepancy cannot be explained by the method of titration used. The increased levels of tryptase in the four groups of patients suggest that mast cells are activated both in allergic and nonallergic rhinitis. This finding appears to be similar to the situation observed in nonallergic asthma in which tryptase levels are increased in bronchoalveolar lavage fluid. Thus although mast cells appear to be involved in chronic rhinitis, the titration of mast cell–derived mediators released in nasal secretions might not be totally adequate to explain the mechanisms of the nasal allergic reaction.

The role of neutrophils in allergic rhinitis remains to be clarified. These cells are usually increased when lavages are carried out 3 to 8 hours after challenge, but they are present both in patients with and without a late-phase reaction, and the presence of neutrophils is not discriminative.[5, 6, 22-24] These cells are also often observed in seasonal allergic rhinitis and in noninfectious perennial rhinitis,[27, 34, 72] and in the present study neutrophils were mainly increased in the nonallergic group (NA S+ and NA S−). Myeloperoxidase is released by activated neutrophils and was found to be increased in many patients of all four groups without any difference between the groups. Taken together, neutrophils may be involved in perennial rhinitis, but the significance of these findings needs clarification.

Other vasoactive mediators including PGD_2 and sulfidopeptide leukotrienes are also released during a nasal challenge.[3, 4, 7-12, 23] Although they do not possess all the vasoactive activities of histamine, particularly the sensitory nerve stimulation, they appear critical

*References 18, 28, 29, 34, 36, 37, 40, 70.

to the allergic reaction, and PGD_2 was found to correlate better than histamine with the occurrence of symptoms during nasal challenge.[7] These two mediators were increased in the A S+ group only when compared with normal subjects, and LTC_4/D_4 may be of importance because this was the second mediator of the study to be increased in the symptomatic group. This finding accords with data observed in seasonal rhinitis where sulfidopeptide leukotrienes were increased during the pollen season.[26, 30] Sulfidopeptide leukotrienes are synthetized by eosinophils,[72, 73] and these increased levels may be related with eosinophil activation as suggested in the present study by the significant correlation between LTC_4/D_4 and EPX levels in patients with rhinitis.

In conclusion, this study suggests a prominant role for the eosinophil in the inflammatory reactions occurring in patients with chronic rhinitis. The titration of EPX and eventually LTC_4/D_4 appear to be valuable tools in the characterization of such patients.

REFERENCES

1. Pipkorn U. Mediators and nasal allergy. Clin Exp Allergy 1989;19:585-9.
2. Walden SM, Proud D, Bascom R, et al. Experimentally induced nasal allergic responses. J ALLERGY CLIN IMMUNOL 1988;81:940-9.
3. Naclerio RM, Meier HL, Kagey-Sobotka A, et al. Mediator release after nasal airway challenge with allergen. Am Rev Respir Dis 1983;128:597-602.
4. Naclerio RM, Proud D, Togias A, et al. Inflammatory mediators in late antigen-induced rhinitis. N Engl J Med 1985;313:65-70.
5. Bascom R, Pipkorn U, Lichtenstein LM, Naclerio RM. The influx of inflammatory cells into nasal washings during the late response to antigen challenge. Effect of systemic steroid pretreatment. Am Rev Respir Dis 1988;138:406-12.
6. Iliopoulos O, Proud D, Adkinson NF Jr, et al. Effects of immunotherapy on the early, late, and rechallenge nasal reaction to provocation with allergen: changes in inflammatory mediators and cells. J ALLERGY CLIN IMMUNOL 1991;87:855-66.
7. Lebel B, Bousquet J, Morel A, Chanal I, Godard P, Michel FB. Correlation between symptoms and the threshold release of mediators in nasal secretions during nasal challenge with grass pollen grains. J ALLERGY CLIN IMMUNOL 1988;82:869-77.
8. Miadonna A, Tedeschi A, Leggieri E, et al. Behavior and clinical relevance of histamine and leukotrienes C_4 and B_4 in grass pollen-induced rhinitis. Am Rev Respir Dis 1987;136:357-62.
9. Shaw RJ, Fitzharris P, Cromwell O, Wardlaw AJ, Kay AB. Allergen-induced release of sulfidopeptide leukotrienes (SRS-A) and LTB_4 in allergic rhinitis. Allergy 1984;40:1-6.
10. Creticos PS, Peters SP, Adkinson NF Jr, et al. Peptide leukotriene release after antigen challenge in patients sensitive to ragweed. N Engl J Med 1984;310:1626-30.
11. Bisgaard H, Robinson C, Rømeling F, Mygind N, Church M, Holgate ST. Leukotriene C_4 and histamine in early reaction in the nose. Allergy 1988;43:219-27.
12. Naclerio RM, Baroody FM, Togias AG. The role of leukotrienes in allergic rhinitis: a review. Am Rev Respir Dis 1991;143:S91-5.
13. Baumgarten CR, Nichols RC, Naclerio RM, Proud D. Concentrations of glandular kallikrein in human nasal secretions increase during experimentally induced allergic rhinitis. Am Rev Respir Dis 1986;137:1323-8.
14. Castells M, Schwartz LB. Tryptase levels in nasal-lavage fluid as an indicator of the immediate allergic response. J ALLERGY CLIN IMMUNOL 1988;82:348-55.
15. Juliusson S, Holmberg K, Baumgarten CR, Olsson M, Enander I, Pipkorn U. Tryptase in nasal lavage fluid after local allergen challenge. Relationship to histamine levels and TAZME-esterase activity. Allergy 1991;46:459-65.
16. Linder A, Venge P, Deuschl H. Eosinophil cationic protein and myeloperoxidase in nasal secretion as markers of inflammation in allergic rhinitis. Allergy 1987;42:583-90.
17. Bascom R, Pipkorn U, Proud D, et al. Major basic protein and eosinophil-derived neurotoxin concentrations in nasal-lavage fluid after antigen challenge: effect of systemic corticosteroids and relationship to eosinophil influx. J ALLERGY CLIN IMMUNOL 1989;84:338-46.
18. Andersson M, Andersson P, Venge P, Pipkorn U. Eosinophils and eosinophil cationic protein in nasal lavages in allergen-induced hyperresponsiveness: effects of topical glucocorticosteroid treatment. Allergy 1989;44:342-8.
19. Bisgaard H, Grønborg H, Mygind N, Dahl R, Lindqvist N, Venge P. Allergen-induced increase of eosinophil cationic protein in nasal lavage fluid: effect of the glucocorticoid budesonide. J ALLERGY CLIN IMMUNOL 1990;85:891-5.
20. Friedman MM, Kaliner M. In situ degranulation of human nasal mucosal mast cells: ultrastructural features and cell-cell associations. J ALLERGY CLIN IMMUNOL 1985;70:70-82.
21. Bascom R, Wachs M, Naclerio RM, et al. Basophil influx occurs after nasal antigen challenge: effects of topical corticosteroid pretreatment. J ALLERGY CLIN IMMUNOL 1988; 81:580-9.
22. Pipkorn U. Involvement of different cell types in allergic rhinitis. In: Pichler WJ, ed. Progress in allergy and clinical immunology. Toronto: Hogrefe & Huber Publishers, 1989:237-41.
23. Meslier N, Braunstein G, Lacronique J, et al. Local cellular and humoral responses to antigenic and distilled water challenge in subjects with allergic rhinitis. Am Rev Respir Dis 1988;137:617-24.
24. Pelikan Z, Pelikan-Filipek M. Cytologic changes in the nasal secretions during the late nasal response. J ALLERGY CLIN IMMUNOL 1989;83:1068-79.
25. Linder A, Strandberg K, Deuschl H. Histamine concentrations in nasal secretion and secretory activity in allergic rhinitis. Allergy 1987;42:126-34.
26. Volovitz B, Osur SL, Bernstein JM, Ogra PL. Leukotriene C_4 release in upper respiratory mucosa during natural exposure to ragweed in ragweed-sensitive children. J ALLERGY CLIN IMMUNOL 1988;82:414-8.
27. Pipkorn U, Karlsson G, Enerback L. Nasal mucosal response to repeated challenges with pollen allergen. Am Rev Respir Dis 1989;140:729-36.
28. Andersson M, Svensson C, Andersson P, Pipkorn U. Objective monitoring of the allergic inflammatory response of the nasal mucosa in patients with hay fever during natural allergen exposure. Am Rev Respir Dis 1989;139:911-4.
29. Svensson C, Andersson M, Persson CG, Venge P, Alkner U, Pipkorn U. Albumin, bradykinins, and eosinophil cationic protein on the nasal mucosal surface in patients with hay fever

during natural allergen exposure. J ALLERGY CLIN IMMUNOL 1990;85:828-33.
30. Skoner DP, Lee L, Doyle WJ, Boehm S, Fireman P. Nasal physiology and inflammatory mediators during natural pollen exposure. Ann Allergy 1990;65:206-10.
31. Gomez E, Corrado OJ, Baldwin DL, Swantson AR, Davies RJ. Direct in vivo evidence of mast cell degranulation during allergen-induced reactions in man. J ALLERGY CLIN IMMUNOL 1986;78:637-45.
32. Enerbäck L, Pipkorn U, Granerus G. Intraepithelial migration of nasal mucosal mast cells in hay fever. Int Arch Allergy Appl Immunol 1986;80:44-51.
33. Viegas M, Gomez E, Brooks J, Gatland D, Davies RJ. Effect of the pollen season on nasal mast cells. BMJ 1987;294: 414-5.
34. Pipkorn U, Karlsson G, Enerback L. The cellular response of the human allergic mucosa to natural allergen exposure. J ALLERGY CLIN IMMUNOL 1988;82:1046-54.
35. Binder E, Holopainen E, Malmberg H, Salo OP. Clinical findings in patients with allergic rhinitis. Rhinology 1984;22:255-60.
36. Jacobs RL, Freedman PM, Boswell RN. Nonallergic rhinitis with eosinophilia (NARES syndrome). Clinical and immunologic presentation. J ALLERGY CLIN IMMUNOL 1981;67:253-62.
37. Mullarkey MF, Hill JS, Webb DR. Allergic and nonallergic rhinitis: their characterization with attention to the meaning of nasal eosinophilia. J ALLERGY CLIN IMMUNOL 1980;65:122-6.
38. Anggard A. Vasomotor rhinitis: pathophysiological aspects. Rhinology 1979;17:31-5.
39. Mikaelian-AJ. Vasomotor rhinitis. Ear Nose Throat J 1989;68:207-10.
40. Mygind N. Nasal allergy. Oxford: Blackwell Scientific Publications, 1978.
41. Malmberg H, Middleton E Jr, Holopainen E, Withl JA. Eosinophilia. In: Mygind N, Weeke B, eds. Allergic and vasomotor rhinitis: clinical aspects. Copenhagen: Munksgaard, 1986:91.
42. Wilson J, Reilly K, Salter D, et al. Nasal histamine and heparin in chronic rhinitis. Ann Otol Rhinol Laryngol 1988;97:389-92.
43. Gleich GJ, Loegering DA. Immunobiology of eosinophils. Ann Rev Immunol 1984;2:429-59.
44. Venge P, Hakansson L, Peterson CGB. Eosinophil activation in allergic disease. Int Arch Allergy Appl Immunol 1987;82:333-8.
45. Olsson I, Venge P, Spitznagel JK, Lehrer RI. Arginine-rich cationic proteins of human eosinophil granules. Comparison of the constituents of eosinophilic and neutrophilic leukocytes. Lab Invest 1977;36:493-500.
46. Uvnas B, Abora CH, Begendorff A. Storage of histamine in mast cells. Evidence for a binding of histamine to protein carboxyls in the granule heparin-protein complex. Acta Physiol Scand 1970;336:1-26.
47. Craig SS, Schwartz LB. Tryptase and chymase, markers of distinct types of human mast cells. Immunol Res 1989;8:130-48.
48. Irani AA, Schechter NM, Craig SS, De Blois G, Schwartz LB. Two types of human mast cells have distinct neutral protease compositions. Proc Natl Acad Sci U S A 1988;140:3936-42.
49. Schwartz LB, Lewis RA, Austen KF. Tryptase from human pulmonary mast cells. Purification and characterization. J Biol Chem 1981;256:11939-43.
50. Klebanoff SJ. Phagocytic cells: products of oxygen metabolism. In: Gallin JI, Goldstein IM, Snyderman R, eds, Inflammation, basic principles and clinical correlates, New York: Raven Press, 1988:391-444.
51. Paterson NAM, Wasserman SI, Said JW, Austen KF. Release of chemical mediators from partially purified human lung mast cells. J Immunol 1976;117:1356-62.
52. Holgate ST, Burns GB, Robinson C, Church MK. Anaphylactic- and calcium-dependent generation of prostaglandin D_2 (PGD_2), thromboxane B_2 and other cyclooxygenase products of arachidonic acid by dispersed human lung mast cells. J Immunol 1984;133:2138-44.
53. Pipkorn U, Karlsson G, Enerbäck L. A brush method to harvest cells from the nasal mucosa for microscopic and biochemical analysis. J Immunol Methods 1988;112:37-42.
54. Smith TF, Remigio LK. Histamine in nasal secretions and serum may be elevated during viral respiratory tract infections. Int Arch Allergy Appl Immunol 1982;67:380-3.
55. Bousquet J, Maasch HJ, Martinot B, et al. Double-blind placebo-controlled immunotherapy with mixed grass pollen allergoids. II. Comparison between parameters assessing the efficacy of immunotherapy. J ALLERGY CLIN IMMUNOL 1988;82:439-46.
56. Onorato J, Merland N, Terral C, Michel FB, Bousquet J. Placebo-controlled, double-blind food challenge in asthma. J ALLERGY CLIN IMMUNOL 1986;78:1140-6.
57. Bousquet J, Calvayrac P, Guérin B, et al. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. I. In vivo and in vitro parameters after a short course of treatment. J ALLERGY CLIN IMMUNOL 1985;76:734-44.
58. Pipkorn U, Karlsson G. Methods for obtaining specimens from the nasal mucosa for morphological and biochemical analysis. Eur J Respir Dis 1988;1:856-62.
59. Carlson M, Hakansson L, Peterson C, Stalenheim G, Venge P. Secretion of granule proteins from eosinophils and neutrophils is increased in asthma. J ALLERGY CLIN IMMUNOL 1991;87:27-33.
60. Peterson CGB, Venge P. Purification and characterization of a new cationic protein-eosinophil protein-x (EPX)- from granules of human eosinophils. Immunology 1983;50:19-26.
61. Wenzel S, Irani AM, Sanders JM, et al. Immunoassay of tryptase from human mast cells. J Immunol Methods 1986;86:139-42.
62. Schwartz LB, Bradford TR, Lee DC, Chlebowski JF. Immunological and physiological evidence for confirmation changes occurring on conversion of human mast cell tryptase from active tetramer to inactive monomer: production of monoclonal antibodies recognizing active tryptase. J Immunol 1990;144:2304-11.
63. Enander I, Matsson P, Nystrand J, et al. A new radioimmunoassay for human mast cell tryptase using monoclonal antibodies. J Immunol Methods 1991;138:39-46.
64. Schwartz LB, Lewis RA, Austen KF. Tryptase from human pulmonary mast cells. Purification and characterization. J Biol Chem 1981;256:11939-43.
65. Morel AM, Delaage MA. Immunoanalysis of histamine through a novel chemical derivatization. J ALLERGY CLIN IMMUNOL 1988;82:646-54.
66. Mc Bride PT, Kaliner MA. Histamine determination: immunologic methods. ACI News 1989;2:50-4.
67. Andersson M, Nolte H, Olsson M, Skov P, Pipkorn U. Measurement of histamine in nasal lavage fluid: comparison of a glass fiber-based fluorometric method with two radioimmunoassays. J ALLERGY CLIN IMMUNOL 1990;86:815-20.
68. Maclouf J, Corvazier E, Wang Z. Development of a radioimmunoassay for prostaglandin D_2 using an antiserum against 11-methoxime prostaglandin D_2. Prostaglandins 1986;31:123-30.

69. Pradelles P, Grassi J, Maclouf J. Enzyme immunoassay of eicosanoids using acetylcholinesterase from electric eel: an alternative to radioimmunoassay. Anal Chem 1985;57:1170-5.
70. Spector SL, English G, Jones L. Clinical and nasal biopsy response to treatment of perennial rhinitis. J ALLERGY CLIN IMMUNOL 1980;66:129-37.
71. Fokkens WJ, Holm AF, Rijntjes E, Mulder PG, Vroom TM. Characterization and quantification of cellular infiltrates in nasal mucosa of patients with grass pollen allergy, non-allergic patients with nasal polyps and controls. Int Arch Allergy Appl Immunol 1990;93:66-72.
72. Bruynzeel PLB, Kok PTM, Hamelink ML, et al. Exclusive leukotriene C_4 synthesis by purified human eosinophils induced by opsonized zymosan. FEBS Lett 1985;189:350-4.
73. Owen WF, Soberman RJ, Yoshimoto T, Sheffer AL, Lewis RA, Austen KF. Synthesis and release of leukotriene C_4 by human eosinophils. J Immunol 1987;138:532-8.

Allergenicity of peanut and soybean extracts altered by chemical or thermal denaturation in patients with atopic dermatitis and positive food challenges

A. Wesley Burks, MD, Larry W. Williams, MD, Wayne Thresher, PhD,[a] Cathie Connaughton, BS, Gael Cockrell, BS, and Ricki M. Helm, PhD
Little Rock, Ark., and Fort Wayne, Ind.

Peanuts and soybeans are two of the six most common foods to cause food hypersensitivity reactions in children. We used the serum of 10 patients with atopic dermatitis and positive double-blind, placebo-controlled, food challenges to peanut and two patients with atopic dermatitis and positive double-blind, placebo-controlled, food challenges to soybean to investigate the change in IgE-specific and IgG-specific binding to these proteins altered by either chemical or thermal denaturation. We used IgE- and IgG-specific ELISA-inhibition analyses to compare these effects on the crude peanut and crude soy extracts, as well as on the major allergenic fractions of both proteins. Heating the soy proteins at various temperatures and time intervals did not significantly change the IgE- or IgG-specific binding of the soy positive pooled serum. When the peanut proteins were subjected to similar heating experiments, the IgE- and IgG-specific binding did not change. When these same proteins were treated with enzymes in the immobilized digestive enzyme assay system used to mimic human digestion, the binding of IgE to the crude peanut and crude soy extracts was reduced; 100-fold for peanut and 10-fold for soybean. Therefore it appears that thermal denaturation of peanut and soybean protein extracts does not enhance or reduce IgE- and IgG-specific binding activity. Chemical denaturation appears to minimally reduce the binding of these proteins. (J ALLERGY CLIN IMMUNOL 1992;90:889-97.)

Key words: *Peanut allergens, soybeam allergens, food hypersensitivity, atopic dermatitis*

From the Division of Pediatric Immunology and Allergy, Department of Pediatrics, University of Arkansas for Medical Sciences, and Arkansas Children's Hospital, Little Rock, and Central Soya, [a]Fort Wayne.
Supported in part by National Institutes of Health grant no. AI26629-03 and Arkansas Science and Technology Authority grant no. 90-A-04.
Received for publication April 2, 1991.
Revised June 25, 1991.
Accepted for publication July 8, 1992.
Reprint requests: A. Wesley Burks, MD, Arkansas Children's Hospital, 800 Marshall St., Little Rock, AR 72202.
1/1/40880

Multiple allergens have been identified in the past several years that stimulate IgE-mediated disease in humans. The identification and purification of these allergens is essential for further studies to understand and characterize the immune response to these antigens.[1] Structural studies of these allergens is also critical to the understanding of the IgE-mediated response.[2] Several inhaled allergens have been characterized from a wide variety of sources, including dust mites, pollens, animal danders, insects, and fungi. Only recently have food allergens been studied with